# Control of *Ixodes scapularis* (Acari: Ixodidae) with topical self-application of permethrin by white-tailed deer inhabiting NASA, Beltsville, Maryland

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ABSTRACT: We report the first successful area-wide reduction of *Ixodes scapularis* by using minimal amounts of permethrin self-applied by free-ranging white-tailed deer in as little as 3 y of nearly continuous treatment. The

study to control all active stages of *I. scapularis* Say was initiated in April 1995, at the Goddard Space Flight Center, National Aeronautics and Space Administration (NASA), Beltsville, Maryland (treated location), and the Patuxent Wildlife Research Center, Laurel, Maryland (non-treated location). The locations had similar flora and fauna, and pre-treatment sampling (April to October 1995) of deer, plots, and mice for *I. scapularis* indicated nearly similar tick populations at both locations. After pre-treatment sampling, 4 deer '4-poster' stations were placed at NASA, while the control area received none. Ten percent permethrin, supplied to 4 roller covers on each station, was passively transferred to the head, neck, and ears of free-ranging deer feeding at the stations. This treatment resulted in elimination of adult *I. scapularis* on sampled deer (100% control) by the 2<sup>nd</sup> y of treatment and reductions of immature tick stages on mice. During the 3<sup>rd</sup> y of treatment, adult, nymphal, and larval questing ticks were reduced by 91-100% from sampled plots, and nymphal and larval ticks were reduced by 70-95% on sampled

Keyword Index: Tick control, deer ticks, deer ectoparasites, '4 Poster', Lyme disease.

#### INTRODUCTION

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In the northeastern and north-central U.S., tick-borne pathogens that cause Lyme disease, human granulocytic ehrlichiosis, and babesiosis are transmitted to humans by the black-legged tick, *Ixodes scapularis* Say. The incidence of these diseases has increased almost every year (Centers for Disease Control 2001), presumably caused by frequent interactions among humans, deer, and infected ticks in the same areas during the same time

periods, usually spring through autumn.

Methods to control ticks include area-wide dispersal of insecticides (Schluze et al. 1984, Solberg et al. 1992, Battaly and Fish 1993, and Stafford 1997), which is effective though poses adverse effects on non-target organisms. Habitat modification, such as burning of

underbrush, may initially control ticks but is rarely used

because it is environmentally destructive. Elimination of deer (Wilson et al. 1988, Stafford 1993, Deblinger et al. 1993) is not well accepted by the public. Close mowing also helps control ticks, but many people bitten in endemic areas are bitten by *I. scapularis* from their own close-cut lawns (Falco and Fish 1988). New approaches to control ticks that are environmentally friendly, acceptable to local communities, more efficacious, and less expensive are needed.

One new approach is the self-application of small amounts of acaricide to free-ranging deer using a self-application system. Several different prototypes of the system were developed, briefly tested, and shown to be effective for decreasing ticks on domesticated goats (Sonenshine 1991), pastured deer (Duncan and Monks 1992), and 11 free-ranging white-tailed deer (Sonenshine et al. 1996). The '4-poster' deer station (Figure 1),

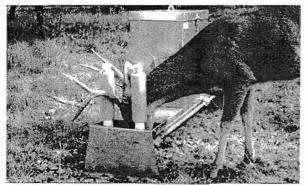


Figure 1. A deer feeding and passively applying acaricide from '4-Poster' Feeding and Application Station.

patented by Pound et al. (1994), was tested in preliminary studies using 1% permethrin self-applied by captive, penned deer, targeting *Amblyomma americanum* in Texas (TX) during 1994 (J.M. Pound and J.A. Miller, unpublished data). A 70% decrease in adult ticks on the treated deer was reported during the 2-mo test period.

The objectives of the present study were to: (1) determine if '4-Poster' deer stations, baited with corn and supplied with the insecticide permethrin, would attract and self-treat the majority of free-ranging deer with small amounts of permethrin (<30 gm Active Ingredient (AI)/deer/yr); (2) evaluate treatment efficacy as measured by an area-wide reduction (≥90%) of all active stages of *I. scapularis* both on and off deer, over a 3-y treatment period.

#### MATERIALS AND METHODS

#### Locations

This research was conducted in Prince George's County, Maryland. Two locations were selected for the study: Goddard Space Flight Center, National Air Space Administration (NASA), Main campus, Greenbelt, MD, and Patuxent Wildlife Research Center, Central Tract, Laurel, MD. These areas were selected because they had similar climate, flora, fauna, and topography. It also was reasonable to assume that tick species and populations, densities of deer and mice, and tick loads (number of ticks parasitizing hosts) would be similar. Both areas were either all (NASA) or mostly (Patuxent) enclosed by fencing, separated by several well-traveled roads, and 2.5 km apart, so it was unlikely that deer would move from one study site to another.

NASA was designated as the treated area because it was more secure, being entirely enclosed by a 2.6 m high security fence topped with 3 strands of double-angled barbed wire, even though the pre-selection of the treatment location had a 50-50 chance of introducing a

source of bias. Patuxent was designated as the non-treated control area. It was enclosed on 3 sides by a 1.8-2.1 m high chain-link fence and on the 4th side by the Patuxent River. The locations differed in area, NASA being approximately 2.55 km² total with approximately 1.21 km² of usable deer habitat, while Patuxent totaled 10.1 km², with 5.3 km² of usable deer habitat. Pre-treatment sampling for all active stages of ticks was conducted in 1995, while post-treatment sampling was conducted from 1996-1998.

Research was conducted in compliance with the Animal Welfare Act and other Federal and Maryland state statutes and regulations relating to animals and experiments involving animals and adhered to principles as stated in the "Guide for the Care and Use of Laboratory Animals," NRC Publications, 1996 edition. All procedures were reviewed and approved by the Animal Care and Use Committee (University of Maryland, College Park, MD), Protocol Number R-95-59

#### Sampling deer for ticks

Ticks parasitizing deer at NASA were compared to those at Patuxent during pre- and post-treatment for a total of 4 y. Six to 12 deer were sampled at each location August to December from 1995 through 1998 for autumn sampling, and March to June from 1996 through 1998 for spring sampling. Only autumn data for each year were shown because only these data were collected for the pre-treatment period. In addition, only infestation of adult ticks was reported because the majority of deer were sampled when immature ticks were not active.

Deer were immobilized by an intramuscular injection of 6 mg/kg xylazine hydrochloride administered using a 1 or 2 cc rifle-fired dart (Pneu-Dart, Williamsport, PA). Immobilized deer were blindfolded to help prevent struggling. A color-coded numbered ear tag (Allflex<sup>TM</sup> USA, DFW Airport, TX) was attached to the right ear of each sampled deer at NASA and to both ears at Patuxent. The sex and age, based on tooth examination (Giles 1978) of the deer, were estimated and recorded. Only the head, ears, neck, chest, and front axillary regions of deer were checked for the presence of ticks by close visual observation and running fingers carefully through the coat in a systematic manner. These areas of the deer contain the majority of all ticks (Schmidtmann et al. 1998), and since we were limited somewhat in time by the deer's reaction to the anesthesia, we checked the most abundant areas. All ticks were identified to species, stage, and sex, using a hand lens (10 x) and samples of adult I. scapularis were returned to the laboratory for microscopic confirmation of identification using the

guide by Keirans and Litwak (1989). Deer were generally revived within 20 min with a 3-5 cc intravenous injection of 0.2 mg/kg of yohimbine hydrochloride. Deer usually regained full motor control within 2-5 min and quickly left the capture area.

# Sampling plots for ticks

Fifteen sampling grids, 250 m<sup>2</sup>, were selected at random in wooded or ecotone areas from both sites. Initially 2- 10 x 10 m<sup>2</sup> (100 m<sup>2</sup>) plots were randomly selected per grid. Each corner of the plot was marked, and the entire plot was dragged to determine the numbers of questing ticks per plot. A 1 m<sup>2</sup> drag (Sonenshine 1991) was placed in a corner of the plot and was pulled slowly through/over the underbrush to an adjacent corner (row 1). The drag was carefully moved over 1 m and pulled adjacent (row 2) to the first drag path. Ticks on the drag were counted after each 2 rows, identified with a hand lens (10x), and a sample was taken back to the laboratory for microscopic confirmation of identification. The remaining ticks were removed from the drag and returned to the center of the rows just dragged. Where larvae

number multiplied by 4 for an estimated count. After sampling, if numerous larvae were still on the drag, the drag was inverted over a shrub or small tree limb and left hanging overnight. Another identical drag was used for the next plot. Ticks were processed in the laboratory as discussed above. A minimum of 2 plots per location was dragged weekly unless prevented by rain or unavoidable circumstances from April through July 15 (Figure 2), when all tick stages were active. No autumn

were too numerous to count, the whole plot was dragged,

larvae on one quarter of the drag were counted and the

# Sampling mice for ticks

Sherman live-capture box traps (7.6 by 8.9 by 22.9 cm; Sherman Traps, Inc., Tallahassee, Florida) were set weekly from June 1 to August 15th, immediately after dragging the test plots (Figure 2). During 1995, 4 traps

drag sampling was conducted for adult ticks.

Timelines

were set at each plot 10 x 10 m plot. During 1996-1998,

4 to 8 traps were set at or near each plot (within 15 m

from the center of the plot) to try to improve trapping

success. The same numbers of traps were set at NASA

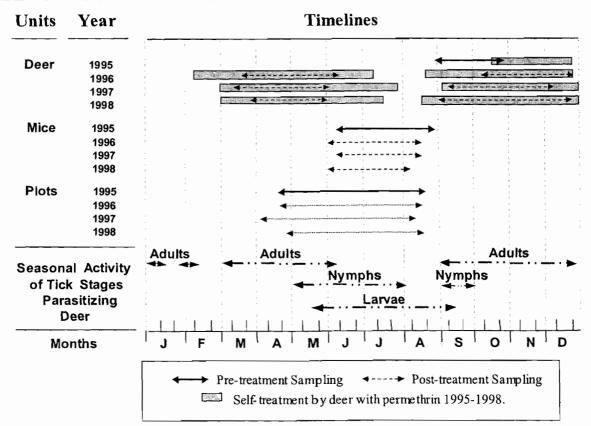


Figure 2. Sampling, permethrin treatment, and seasonality time lines.

and Patuxent during the same week except when occasional rain prevented trapping. The traps were baited with peanut butter and set between 1800 to 2000 (EST) hours and checked the next morning between 0600 to 0800 hours. Each trapped mouse (15-20 gms) was anesthetized at the time of capture with a mixture of ketamine (10 mg/ml): Rompum (100 mg/ml): sterile water (2.5:0.5:8.0 ml) to an effective light level of anesthesia. Each mouse was identified to species using the Audubon Society Field Guide to North American Mammals (Whitaker 1980). Larval and nymphal ticks were counted on the mouse and a sample of ticks taken back to the laboratory for confirmation of identity. Each mouse was sexed, the data recorded, and the mouse then released at the point of capture once activity returned.

#### '4-Poster' deer stations

We used 22.9 cm long paint roller covers on the roller holders of each station (Figure 1). During the first two years, foam-type paint roller (Slick Foam Roller Cover, Model/style 07040, 2.1 cm foam; Shur-line, Inc., Lancaster, NY) were used. The last two years the covers were switched to sturdier fiber-type paint covers (Pylan Synthetic Lambskin, extra rough surface, Synthetic Lambskin, 3.2 cm nap; Linzer Products Corp., Flushing, NY) because large chunks of foam were being striped from the covers. The deer stations were placed 10-15 m inside wooded areas, where deer were more secluded and would feed more readily. The feed reservoir of each station was filled with 225 lb (113.5 kg) of whole, shelled corn. To initially attract deer to the stations, extra corn and apple slices were dispersed along trails leading to the stations, at the bases of the stations, and on the stations. The extra bait was discontinued once >1 deer was observed feeding at that station. All other vertebrates eating corn or corn fines (small pieces of corn) were recorded while re-supplying the deer stations.

The insecticide initially used to treat the deer was Synergized Expar Pour-On (active ingredient: 1% permethrin, EPA Registration No. 59-229, Mallinckrodt Vet Inc., Mundelein, IL). Permethrin is a residual insecticide used to kill ectoparasites on domesticated animals. Because this formulation is approved for use on dairy and beef cattle for the control of ectoparasites, the EPA issued a research waiver for use on non-hunted deer at NASA. Fifteen ml of permethrin was applied as evenly as possible via a measured squeeze bottle to each roller, 5 d/wk, for the first month of treatment. After one month, we switched to a higher percentage of permethrin formulation (10% permethrin, Brute<sup>R</sup>, EPA Reg. No. 89039-7, Y-Tex, Inc. Cody, WY). It was difficult to determine the numbers of deer being treated, so a nontoxic dye, normally used to highlight human eyes, was

added to the permethrin (1 gm D&C Green Dye #6 [Tri-Con Colors Inc., Elmwood Park, NJ]/476 ml Brute) to mark the treated deer. Fifteen ml of the permethrin-dye solution were applied to the rollers from mid-November 1995 through December 1998. The rollers generally were treated with the solution 3 times/wk for 8 weeks during 1995, 2.5 times/wk for 9-10 months in 1996-97, and 2 times every other week for 9-10 months in 1998 (Figure 2).

The stations were closed periodically by sliding the trough plates across the feeding troughs and screwing them tightly to the frames. Such closures were effective before and during inclement weather to prevent wetting, molding, and caking of corn fines and clogging of the feeding chute. After inclement weather, permethrin was added to the rollers and the stations were opened for the period scheduled. The stations were also closed when tick stages were inactive during freezing weather in January, February, and part of March; at the end of August and the early part of September when tick activity was low; and during intervals of several days to 1 wk throughout the year to limit permethrin application.

During June 1996, there were about 100 Canadian geese (*Branta canadensis*) at NASA. Several geese started feeding from the stations with the deer, and soon 30 geese were counted feeding from all 4 stations. To prevent the geese intrusion, each feeder was placed on four 15.2 W x 45.7 H x 61.0 L cm concrete blocks. The stations were left on the blocks throughout the study.

#### Deer counts

Because deer densities influence adult tick densities (Deblinger et al. 1993), deer densities per usable habitat acre (excluding ponds, swamps, buildings, parking lots, etc.) were estimated for both locations. Two types of counting methods were used: direct visual counts

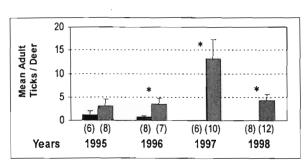


Figure 3. Mean ( $\pm$  SEM) adult *Ixodes scapularis* sampled from deer at NASA and Patuxent. Numbers in parentheses under the bars indicate sampled deer at NASA (solid black bars) and Patuxent (striped bars). Yearly significant differences(\*) occurred between locations (1-tailed, 2-sampled T-test) for the following years: 1996 (T=2.01, df=6, P=0.0450); 1997 (T=3.06, df=9, P=0.0068); and 1998 (3.82, df=11, P=0.0014).

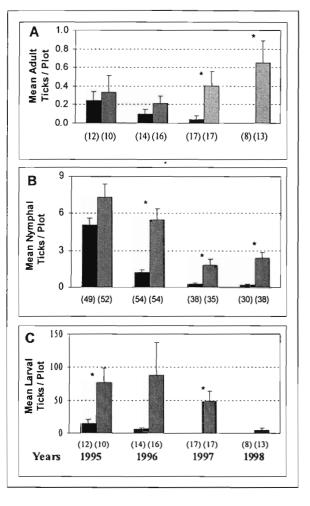


Figure 4. Mean (± SEM) questing *Ixodes scapularis* sampled from test plots. Numbers in parentheses indicate numbers of sampled plots at NASA (solid black bars) and Patuxent (striped bars). The sampling years are shown at the bottom of the graph. An asterisk (\*) above the bars indicates a significant difference between locations.

A. Yearly significant differences between adult ticks collected from the two locations occurred for the following years (1-tailed, 2-sampled T-test): 1997 (T=2.13, df=27, P=0.0210); and 1998 (T=2.71, df=22, P=0.0063).

B. Yearly significant differences between nymphal ticks collected from the two locations occurred for the following years (1-tailed, 2-sampled T-test): 1996 (T=4.92, df=59, P<0.0001); 1997 (T=3.43, df=38, p=0.0007); and 1998 (T=4.87, df=49, P<0.0001).

C. Yearly differences between larval ticks collected from the two locations occurred for the following years (2-tailed 2-sampled T-test for 1995, 1-tailed for the other years): 1995 (T=2.53, df=10, P=0.0300); 1996 (T=1.64, df=15, P<0.0600); 1997 (T=3.07, df=16, P=0.0037); and 1998 (T=1.61, df=12, P<0.0660).

conducted by a walking drive-line census at NASA (DeCalesta and Witmer 1990, Penland 1999), and an area strip census involving direct observation from motor vehicles driving a standard route in Patuxent at dusk (Cockrum 1962, Deblinger et al. 1993).

#### Quantification of insecticide residue on deer

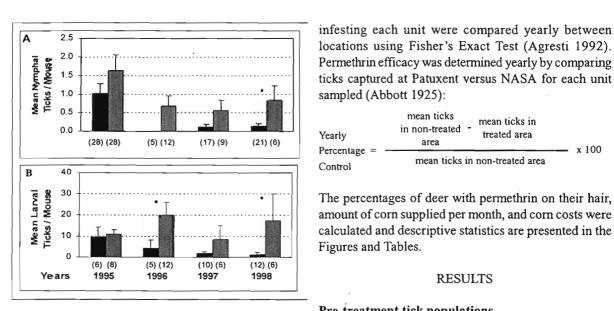
To estimate the number of deer using the deer stations each year, each sampled deer was first evaluated visually for dye on the head, ears, and neck. A 1-2 cm<sup>2</sup> section of hair was cut near the skin from the poll of the head, center of the neck, and upper leg, on 1 side of the deer. In 1996, the sampling area was changed to the junction of the head and neck since that was where the permethrin was mainly deposited (Figure 1). The samples were placed into 20 ml glass scintillation vials and returned to the laboratory to determine the presence of permethrin by high pressure liquid chromatography (HPLC) analysis (Miller et al. 1983). Briefly, 0.4 g of hair was placed in 20 ml acetonitrile in a scintillation vial. The vial was placed on a shaker/roller for 24 h to allow the concentration of permethrin on the hair and solvent to reach equilibrium. A C18 column was used in the Waters HPLC system, along with 80:20 acetonitrile:water mobile phase and a flow rate of 1.5 ml/min with a 254 micron wave length. Known permethrin standards were prepared and 201/4 g of each standard was injected into the HPLC system. Then the samples were injected and compared to the standards of known concentrations. Printouts of the peaks, height of the peaks, and the area under the curve were obtained from the standards and unknown hair samples using a Hewlett-Packard model 3390A computing integrator. A graph of the concentrations of the known standards peak heights versus the peak heights of the unknowns was plotted and compared. Values of the unknowns were read directly from the graphs.

#### Statistical methods

Three factors associated with *I. scapularis* activity, (1) ticks parasitizing free-ranging deer, (2) questing ticks collected from test plots by dragging, and (3) ticks parasitizing mice trapped from test plots, were measured and each factor was compared between the treated and non-treated locations over 4 y (pre-treatment, 1995; post-treatment, 1996-1998).

The numbers of mice caught per trap-night were compared between locations for each year. The mean densities of ticks infesting each unit (deer, mice, and plots) were compared yearly between locations using the two sample T-test (MINITAB Statistical Software, PWS-KNT, Boston, MA). A significant level (alpha) <0.05 was considered significant. The percentages of ticks

C4-4:-4:-



A. Yearly differences for nymphal ticks between locations, 1tailed 2-sampled T-test: 1996 (T=2.35, df=11, P=0.0390) and 1998 (T=2.74, df=25, P=0.0110). B. Yearly differences occurred between larval ticks collected

from the two locations, 1-tailed 2-sampled T-test: 1996

(T=2.23, df=14, P=0.0430).

Figure 5. Mean (+ SEM) nymphal and larval Ixodes scapularis

sampled from white-footed mice. Numbers in parentheses

show the number of sampled mice at NASA (solid black bars)

and Patuxent (striped bars) for each year sampled. The

sampling years are shown at the bottom of the graph. An asterisk (\*) above the bars indicates a significant difference.

locations using Fisher's Exact Test (Agresti 1992). Permethrin efficacy was determined yearly by comparing ticks captured at Patuxent versus NASA for each unit sampled (Abbott 1925): mean ticks

mean ticks in in non-treated treated area Yearly агеа x 100 Percentage mean ticks in non-treated area Control

The percentages of deer with permethrin on their hair, amount of corn supplied per month, and corn costs were calculated and descriptive statistics are presented in the Figures and Tables.

# RESULTS

# Pre-treatment tick populations

The mean numbers of adult I. scapularis sampled from non-treated deer at both locations were not significantly different from one another (NASA 3.12±1.5 SEM; Patuxent 1.33±0.67; Figure 3), as were the percentages of deer infested with adult ticks (NASA 50% versus Patuxent, 75%; Table 1) between locations. Mean numbers of adult and nymphal I. scapularis collected from plots by dragging were not significantly different for either stage between locations (Figure 4). The percentage of plots infested with adult ticks showed no

(61.5-99.8)

Table 1. Yearly comparisons between number of deer sampled and percent infested with adult Ixodes scapularis from NASA versus Patuxent.

	NASA (Treated Site)				Patux	ent (Control Site)	Statistics
Pre-TRT/ Post-TRT per Y <sup>1</sup>	T/NT²	No. Deer Infested /Sampled	Percentage of Deer Infested (± 95% CI <sup>3</sup> )		No. Deer Infested /Sampled	Percentage of Deer Infested (± 95% CI)	P Values <sup>4</sup>
Pre-TRT							
1995	NT	3/6	50.0	NT	6/8	75.0	0.5804
			(11.8-88.2)			(34.9-96.8)	
Post-TRT							
1996	T	4/7	57.1	NT	7/7	100.0	0.0962
			(18.4-90.1)			(59.4-100.0)	
1997	T	0/10	0.0	NT	10/10	100.0	< 0.0001
			(0.0-30.9)			(69.2-100.0)	
1998	T	0/8	0.0	NT	11/12	91.7	< 0.0001

Pre-treatment (Pre-TRT) sampling or post-treatment (Post-TRT) sampling per year. <sup>2</sup> Treatment/Nontreatment (T/NT)- application of permethrin or no application of permethrin to deer.

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(0.0-36.9)

<sup>3 95%</sup> Exact Confident Interval (CI).

Fisher's Exact Test; two-tailed P-values were calculated for 1995, one-tailed P-values for 1996-1998.

ole 2. Yearly compariso	n of plots infested w	Table 2. Yearly comparison of plots infested with Ixodes scapularis sampled at NASA versus Patuxent.	at NASA versus P	atuxent.		Jun
		NASA		Patuxent	Statistics	e, 200
Ţ.	Plots Infested	% Plots Infested	Plots	% Plots	Δ	)3
Stage Years T	r¹ /Sampled	(± 95% Exact CI²)	/Sampled	(± 95% Exact CI)	Values	
Adult 1995 NT	Γ 5/21	23.8	4/18	22.2	1.0000	
1996 T	Γ 3/31	(8.2-47.2) 9.7	8/28	( 6.4-47.6) 21.4	0.1868	
		(2.1-26.5)		(8.4-41.0)		

10/25

1/24

1997

0/23

1998

(0.1-21.1)

(0.0-14.8)

65.4

34/52

N

1995

Nymphal

(50.9-78.0)

8/23

46/49

44/54

61.1

33/54

Γ

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Values <sup>3</sup>	
1.0000	
0.1868	
0.0028	
0.0019	
0.0005	
0.0163	
90000	
0.0001	

(21.1-61.3)34.8 (83.1-98.7)

(16.4-57.3)93.9 (8.06-9.89)

54.3

19/35

15.8 (6.0-31.3)15.8 (6.0, 31.3)

82/9

1997

(46.9-74.1)

9000.0	<0.0001	

Ecology	

01	33

(61.2, 87.4)

2.99

8/12

64.3

9/14

NT

1995

Larval

86/38

1998

(36.7-71.2)

76.1

35/46

(34.9-90.1)

56.3 (29.4-80.3)76.5

9/16

(35.1-87.2)

71.4

10/14

Г

1996

(41.9-91.6)

(1.5-36.4)

9.1

1/11

1998

(0.2, 41.3)

13/17

11.8

2/17

1997

E	co	lo	gy

_	
0	~
$\overline{}$	_

0.613

0.0002

0.8931

0.1121

<sup>1</sup> Treatment (T) occurred at NASA, Oct. 1995-Dec. 1998. No treatment (NT) occurred during sampling of plots Apr.-Oct.1995 at NASA; and Patuxent, Apr.

<sup>3</sup> Fisher's Exact Test; two-tailed P-values were calculated for 1995; one-tailed P-values for 1996-1998.

<sup>2</sup> 95% Exact Confident Interval (CI)

1995-Dec. 1998

(15.2, 64.6)

37.5

6/16

(50.1-93.2)

123

significant difference (Table 2), nor did the percentage of larval infested plots during 1995. The only significant pre-treatment differences between locations were the percentage of plots infested with nymphal ticks (Table 2) and the mean number of larval ticks collected per plot (NASA 13.1±5.2, n = 12; Patuxent 63.6±21.0, n=10; Figure 4).

Only *Peromyscus leucopus* were captured during the study. The mean numbers of nymphs and larvae per mouse pre-treatment were not significantly different between NASA and Patuxent, nor was the percentage of mice infested with nymphal and larval ticks at each location (Figure 5, Table 3). The numbers of mice captured at each location were similar (Table 4). Pre-treatment counts of deer at both locations also were similar (Figure 6).

#### Post-treatment tick populations

Complete elimination of all adult *I. scapularis* parasitizing sampled deer at NASA (only adult autumn tick data are shown) was achieved during the 2<sup>nd</sup> and 3<sup>rd</sup> y of deer treatment with permethrin (Figure 3, Table 1), even on those deer sampled without detectible levels of permethrin on their hair. Conversely, deer at Patuxent had significantly higher mean numbers of adult ticks during 1996, 1997, and 1998 (Figure 3). From 1996 to 1998 the percentage of Patuxent deer infested with adults were 100, 100, and 92%, while NASA infestation rates were 57, 0, and 0% (Table 1), respectively.

Overall, mean numbers of questing NASA *I. scapularis* of all stages collected per plot showed a continuing decline to near zero or zero (Figure 4) from 1996 to 1998, and were close to or significantly greater at Patuxent each year (Figure 4). After the second year

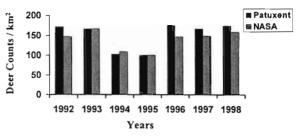
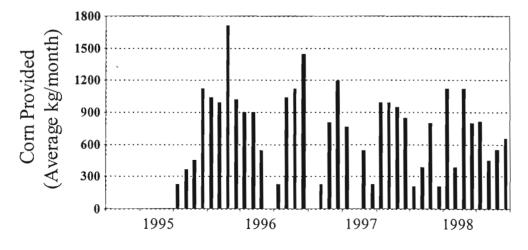


Figure 6. Estimated deer densities at NASA and Patuxent, 1995-1998.

of treatment, adult ticks sampled from plots at NASA declined to zero (Figure 4). Declining mean nymphal ticks per plot showed significant decreases during 1996 to 1998 (Figure 4). Mean numbers of larvae per plot were significantly different between locations in 1997, and approached the significance level in 1996 and 1998 (Figure 4), with more larvae collected at Patuxent than at NASA during each year post-treatment. Larvae showed extreme clumping at Patuxent (ranges: 1995, 0-263; 1996, 0-800; 1997, 0-200; and 1998, 0-37). Less clumping of larvae was noted at NASA (ranges: 1995, 0-72; 1996, 0-19; 1997, 0-3; and 1998, 0-4). Larval infestations decreased to 9.1% of the plots sampled at NASA in 1998 compared to 37.5% at the control area (Table 2).

Mean numbers of nymphal ticks collected from mice at NASA were significantly less than those collected at Patuxent during 1996 and 1998 (Figure 5). During 1997, mean numbers of nymphal ticks per mouse at NASA were also less than those from Patuxent; however, the differences were not significant (Figure 5). The percentage of mice infested, though lower at NASA than



Months

Figure 7. Amount of corn per month provided to deer at NASA, 1995-1998.

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ent.	Statistics <sup>1</sup>	e of sted P
ected from NASA versus Patuxent.	Patuxent	Percentage of Mice Infested
ected fi		Mice ested/

Table 3. Ye	arly compa	rison of <i>l</i>	peromyscus leuco	Table 3. Yearly comparison of Peromyscus leucopus infested with Ixodes scapularis collected from NASA versus Patuxent.	<i>llaris</i> collected from NA	.SA versus Patuxent.		ine, 2
				NASA	Patuxent	cent	Statistics1	.003
Tick Stage	Years	Ē	No. Mice Infested /Sampled	Percentage of Mice Infested (± 95% Exact CI²)	No. Mice Infested/ Sampled	Percentage of Mice Infested (± 95% Exact CI)	P Value³	1
Nymphal	1995	LN	13/28	46.4	19/28	67.9	0.1765	
	1996	Т	9/2	0.0	5/12	(1.75-7.77) 41.7 41.7	0.1280	
	1997	Т	2/17	(0.0-52.2) 11.8 (1 5-36 4)	3/9	(15.2-7.2.3) 33.3 (17.5-70.1)	0.2081	
	1998	Т	3/21	(3.1-36.3)	3/6	(17.57/8.1) 50.0 (11.8-88.2)	0.1009	Journa
Larval	1995	L	9/9	100	8/8	100	1.000	ıl of Ve
	1996	Н	2/5	40.0	11/12	91.7	0.0525	ctor E
	1997	Τ	8/10	80.0	4/6	(6.25-2.10)	0.8819	colog

(54.1-100.0)40.0 (5.3-85.3)80.0 (44.4-97.8)

0.0113 100.0 (99.5-100.0)

'ogy

(22.3-95.7)

9/9

(9.9-65.1)

33.3

4/12

1998

0.0017

1 No treatment (NT) occurred during mouse sampling at NASA, 1995, and Patuxent, 1996-1998. Treatment (T) occurred to deer at NASA during the end of 1995

<sup>3</sup> Fisher's Exact Test; two-tailed P-values were calculated for 1995; one-tailed P-values for 1996-1998.

<sup>2</sup> 95% Confident Intervals (CI).

to 1998.

Year

1995

1996

1997

1998

<sup>2</sup>Z test

Year

1995

1996

1997

1998

times deer counts for each y.

%

**Positive** 

Traps

29.2

4.2

14.7

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Mice caught

per

Trap-night

28/104

17/124

9/174

Patuxent

%

Positive

Traps

26.9

13.7

5.2

5.5

% Tick Control1

1997

100

100

90

86

100 79

Statistic<sup>2</sup>

 $\mathbf{Z}$ 

Value

0.35

-2.66

2.57

3.17

1998

100

100

100

91

92

70

95

Estimated

Deer Treated With Perm<sup>3</sup>

0

30

45

61

June, 2003

P

Value

0.724

0.008

0.010

0.002

Table 4. Mice caught per trap-night from plots at Patuxent versus NASA.

Mice caught

per

Trap-night

28/96

5/120

17/116

Units sampled

(methods)

Deer (Deer)

Plots (Drag)

NASA

21/109	19.3	6/110
nber of traps set	each night).	

Tick

stages

Adults

Adults

Nymphs

Nymphs

<sup>1</sup> Trap-night (numb

Table 5. Area-wide control of *Ixodes scapularis* measured by three sampling methods.

Larvae Plots (Mice) Nymphs Larvae <sup>1</sup>% Control = (Patuxent Mean Ticks - NASA Mean Ticks) x 100, post initiation of permethrin treatment to deer at (Patuxent Mean Ticks)

58 NASA.

0

43

64

83

<sup>3</sup> Estimated number of deer treated with permethrin = percentage of deer sampled with permethrin-treated hair

1996

78

100

55

78

93

100

75

Deer

Count<sup>2</sup>

75

69

70

74

Table 6. Deer hair sampled at NASA for permethrin.

0/9

3/7

7/1

<sup>2</sup> Deer counts were conducted yearly, September to December.

10/12

Number Deer Sampled with

% Deer Perm<sup>1</sup>/Total Sampled with Perm Deer Sampled

<sup>1</sup> Permethrin (Perm) was applied to the first deer station during October, 1995.

applied twice/yr (g AI/ha)

3 See Table 6.

Number of applications/wk

Ą.

control

5. Quantity of Brute/deer/wk

B.

Table 8. Estimated yearly corn and permethrin supplied to deer feeders and costs, and estimated labor costs to maintain the deer feeders.

Year	Corn Supplied/Yr Lbs (kg)	Corn Cost \$/Yr¹	Permethrin Cost/Yr² \$	Labor Cost/Yr \$
1995	4,730 (2,150)	433	103	6,528
1996	23,125 (10,511)	2,070	489	15,300
1997	16,825 (7,647)	1,506	356	10,880
1998	16,300 (7,409)	1,459	345	8,160

<sup>&</sup>lt;sup>2</sup> Based on market value of Brute.

was 100, 79, and 70% (Table 5).

During 1996, mean numbers of larval ticks collected per mouse at NASA were significantly less than those collected at Patuxent (Figure 5). Declining larval populations continued at NASA in 1997 and 1998 but were not significantly different between locations (Figure 5). Nevertheless, by 1998, the percentage of larval ticks infesting mice was significantly less at NASA compared

at Patuxent, was not significantly different, even though

no ticks were found on mice at NASA in 1996 and only

a few in 1997-1998 (Table 3). The percentage control of

nymphs parasitizing mice at NASA from 1996 to 1998

#### Deer densities and sampling Deer were counted each autumn at both locations.

to Patuxent (Table 3).

NASA.

In 1995, walking drive-line deer censuses determined a range of 50-75 deer (n=5) for a total of 630 acres (2.55 km2) at NASA (Penland 1999), or a total of 300 deerusable acres (1.21 km<sup>2</sup>). The maximum number of counted deer at NASA (75) occurred in 1995. NASA and Patuxent had similar deer densities, 100 deer/deer usable 2.6 km<sup>2</sup>, during 1995 (Figure 6). From 1996-1998, the deer densities at both locations increased, with slightly more deer sighted at Patuxent compared to

The NASA location was not as secure for deer as expected. Two to 5 deer per y were observed walking out gates, jumping fences, going through holes in the fence, or standing outside the areas. Generally most deer that left returned, though several remained outside or went elsewhere. Two marked deer were killed by cars outside the fenced perimeter during the study. Deer emigrated from Patuxent also. One ear-tagged Patuxent buck was shot 11 miles north of Patuxent and another was sited 11 miles southwest of Patuxent. However, no Patuxent deer were ever sited at NASA or vice versa.

During the autumns of 1995-1998, a total of 31 deer

while a total of 37 deer were sampled at Patuxent, 8 males and 28 females. The sexes and ages of 3 deer were not recorded. More male deer were sampled at NASA as compared with Patuxent, both in total number and percentages of deer sampled. The numbers and estimated ages of deer captured and recorded were: NASA, 0.5-1.8 y, 13 deer; 2-3.8 y, 11 deer; and  $\geq$ 4 y, 5 deer; Patuxent, 0.5-1.8 y, 15 deer; 2-3.8 y, 12 deer; and  $\geq$  4 y, 4 deer.

were sampled at NASA, 13 male and 16 female deer,

#### Mice captured

The numbers of P. leucopus captured yearly varied significantly between locations from 1996-1998 (Table 4). However, overall number of mice captured/y ranged from 28 to 5 mice at NASA and 28 to 6 mice at Patuxent over the 4 y of trapping.

'4-Poster' usage Deer stations were efficacious in attracting, feeding, and permethrin-treating free-ranging deer (Table 7). Deer that were sampled the greatest distance from all 4poster deer stations had permethrin on their hair, indicating they had traveled ≥1000 m to use a station. Some ear-tagged deer were observed using 1 station at least twice a day and some were observed eating at different stations during the same week. As many as 4 deer fed per station.

While supplying corn and permethrin to the deer stations, other animals were observed eating corn from the stations, including Canadian geese, B. canadensis; gray squirrels, Sciurus carolinensis; gray foxes, Urocyan cinereoargenteus; racoons, Procyon lotor; white-footed mice; eastern chipmunks, Tamias striatus; American crows, Corvus brachyrhynchos; and small unidentified passerine birds.

Chunks of foam roller covers were found missing from the rollers from 1995 to 1996, presumably stripped by small to medium mammals since no foam pieces were

material or eaten. This necessitated replacement of the covers as often as every several days to several weeks until the type of covers was switched to Synthetic Lambskin in 1997, thus eliminating the problem. Even though the purchased corn was re-cleaned by the provider, commeal and corn fines were still present. This necessitated cleaning the deer station chutes and troughs

found. It appeared the foam was either used as nesting

after rains, or the deer would not feed and self-apply

permethrin due to caking and molding of the corn meal.

Eventually we changed corn suppliers and eliminated

dye solution was added to the first station, several small

groups of deer were observed eating corn or foraging

along the roadside with "blue-green" dye on their ears,

#### Detection of permethrin on deer hair In mid-October 1995, soon after the permethrin-

the problem.

indicating self-treatment with the permethrin-dye solution. Nevertheless, none of the deer sampled had visible dye on their head, neck, or ears, nor permethrin on their hair samples in 1995 (Table 6). NASA deer with detectable permethrin levels increased steadily from 1996 (43%) to a minimum of 83% in 1998 (Table 6). No ticks were found on the permethrin-treated deer. During HPLC analysis, the dye eluded first and did not interfere with the permethrin cis- and trans-isomer peaks. Samples as low as 0.6 µg permethrin/gm hair could be detected.

#### Estimated permethrin applied to deer

Based on yearly deer counts and the amount of permethrin added to the stations, a maximum estimate of 20.6 mg permethrin/kg deer/wk was applied during permethrin treatment in 1996 (Table 7). By 1998, the

amount had been reduced to 4.12 mg permethrin/kg deer/

wk (Table 7). The yearly cost based on market value

over the last treatment year was approximately \$345 for

all 4 stations to treat 61-74 deer (Tables 6 and 8).

Corn and permethrin use and costs Corn supplied to the stations increased from 245 kg in September to a high of 1724 kg in March 1996 (Figure 7). Corn was thereafter limited to deer by closing the feeders for 1 to 2 d per wk to a month during selected times. Yearly amounts and costs of corn and permethrin supplied to the stations and estimated labor costs to

maintain the stations are shown in Table 8.

#### DISCUSSION

Control of ticks The insecticide Permethrin was used to control ticks. One application of permethrin on cattle will control horn,

face, stable, horse, house, black, and deer flies, lice, and mosquitoes for approximately 2 wk when applied according to directions (Y-Tex label, Cody, WY). Permethrin has also been reported to have a repellent

effect against some tick species (Lane and Anderson

NASA before 1992 (Penland 1999) and at Patuxent

before 1989 (Amerasinghe et al. 1992, Daniels et al.

1984). Permethrin self-treatment by deer drastically reduced all active stages of the tick population, both on and off deer, throughout the 3-y treatment at NASA (Figures 3-5, Tables 1-3, and 5).

#### Pre-treatment sampling I. scapularis populations were well established at

1993, Schmidtmann et al. 1998). Overall, pre-treatment sampling at both locations was not significantly different in tick populations of all active stages per unit sampled, with the exceptions of percentage of plots infested with nymphal ticks (Table 2) and mean numbers of larvae/ plot (Figure 4C). However, there were no pre-treatment differences in mean nymphal densities/plot (Figure 4B) and percentage of plots infested with larvae (Table 2). These differences may have been caused by chance occurrence of sampling plots with higher spatial aggregations (clumping) at Patuxent and a low number of plots sampled for larvae at both locations. Spatial aggregation of larvae, nymphs, and adults is a wellknown characteristic of I. scapularis (Ginsberg and Ewing 1989, Daniels and Fish 1990, Stafford 1992, Telford et al. 1992, Ostfeld et al. 1996a, b). A replete female tick lays 860-3,000 eggs (Hooker et al. 1912, Rogers 1953, Oliver et al. 1993) near the location where she dropped from the host, and with larvae only traveling 1-2 m from the egg mass, larvae are the highest clumped of all motile stages (Daniels and Fish 1990, Ostfeld et al. 1996a). In 1995, the largest larval clumps were found at Patuxent (range 0-263 larvae per plot), while the range

Post-treatment tick populations Deer are major hosts for adult I. scapularis and also an important blood meal source for immature stages (Wilson et al. 1990). Maximum numbers of 1,000-2,000 larvae and >40-60 nymphs per deer have been reported (Piesman and Spielman 1979, Anderson and Magnarelli 1980, Telford et al. 1988). We found >60 larvae and 21 nymphs parasitizing a deer's head, neck, and chest at Patuxent in June 1996, but not from deer sampled in June at NASA (data not presented).

at NASA was 0-30 larvae/plot.

The clear decreases in immature stages sampled from plots and mice during 1996 (Figures 4-5) also

indicate that the permethrin-treated deer killed nymphal

and larval ticks as well as adult ticks during 1996. If permethrin only killed adult ticks in 1996, a decrease in larval ticks would not be observed until 1997, and nymphal ticks would not decrease until 1998. An early knockdown of immature ticks is important to prevent further transmission of *B. burgdorferi* by nymphal ticks. In addition, though deer are generally considered as systemically incompetent reservoirs of *B. burgdorferi* (Telford et al. 1988), *B. burgdorferi* spirochetes have been transferred experimentally from artificially infected

been transferred experimentally from artificially infected deer to non-infected immature ticks at least 43 d after infection (Oliver et al. 1992), and viable spirochetes have been isolated from an ear injection site of a deer at least 70 d post-infection (Luttrell et al. 1994). The route of transmission may be non-systemic since *B. burgdorferi* transmission between co-feeding ticks of different stages occurs in non-systemically infected rodents (Patrican 1997). Thus it may also be important to kill all stages of ticks parasitizing deer to prevent non-systemic transmission of *B. burgdorferi*.

Though larval sampling showed decreased mean numbers per plot at NASA compared to Patuxent through 1998, the pre-treatment significant difference observed between locations in 1995 obscured the meaning of the significant difference found in 1997 (Figure 4). Additional larval sampling through the end of July may have provided more data and given a more accurate picture over the 4 y of sampling.

The sharp decrease and continued suppression in

the tick population at NASA (Figures 3-5, Tables 1-3 and 5) could only be caused by permethrin treatment of deer, even though the study was a single replication and lacked treatment randomization of the two areas. Lack of randomization was minimized due to similarities in pre-treatment tick sampling at both locations, strong posttreatment effects demonstrated on the units sampled at NASA, and consistency of the treatment effects at NASA over the 3 treatment years. In addition, two additional non-treated control areas, 30 to 500 m outside the NASA security fence, were sampled for nymphal ticks by dragging in 1998 and confirmed significantly higher nymphal populations per plot compared with the NASA population (data not presented). Furthermore, the mean number of nymphal ticks sampled per plot at the new control areas were virtually identical to those at Patuxent in 1998, demonstrating that only the NASA tick population decreased to near zero. At no time did the mean number of adult ticks collected from deer at Patuxent or Ft. Meade, MD in 1999, another area adjoining Patuxent, decrease to or approach zero (J.F.

Carroll, unpublished data), as happened at NASA.

#### Deer densities

Deer densities may affect tick densities<sup>1</sup>. Several years of deer census baseline data at NASA were recorded by the walking drive-line census (Penland 1999), one of the most accurate methods of counting deer within fenced enclosures (DeCalesta and Witmer 1990). A strip census, recommended for larger areas not completely enclosed (Cockrum 1962), was used at Patuxent. Even though these methods were different, it was decided to use the most accurate method for each location.

Deer counts in 1995 indicated that pre-treatment deer densities were similar at both locations. Though deer counts varied from year to year and between locations, the overall population trends between the small deer herds remained roughly similar or slightly higher at Patuxent (Figure 6). The harsh winters of 1993 and 1994 decreased deer densities at both locations in 1994 and 1995, with many winter kills found in the spring months at Patuxent (H. Obrecht, Patuxent Wildlife Research Center, Laurel, MD, personal communication). Small groups (5-10 deer/group) of juvenile and adult deer of both sexes were observed crossing the Patuxent River daily from the Patuxent North Tract into the Central Tract control site to feed and then returning to the North Tract. No daily deer migration was observed at NASA. However, yearly migration of deer was observed at both locations, with greater numbers of deer migrating to and from Patuxent than at NASA. The few deer (1-5/y) that left NASA consisted of either sex and all ages >6 mo. Though the deer counts at Patuxent appeared to be slightly higher than at NASA during the last 3 y, the counts at Patuxent may have reflected more immigration

#### Mice

Counting *I. scapularis* on hosts is the most efficient method of monitoring tick populations when tick densities are low (Lane et al. 1991). Thus, anticipating that our control method would yield low numbers of immature ticks in the treated area toward the end of the study, we included mice as one of our sampling methods. Yearly fluctuations in mouse densities at a given location are the norm, varying as much as tenfold between years at the same trapping sites, caused by such extrinsic factors as climate, mast production, and predation (Ostfeld et al. 1996c). We expected yearly mouse densities at NASA and Patuxent would be somewhat comparable to one

or a less accurate counting method.

<sup>&</sup>lt;sup>1</sup>Ginsberg, H.S. 1992. Ecology and management of ticks and Lyme disease at Fire Island National Seashore and selected national parks. Scientific monograph NPS/NRSUNJ/NRSM-92/20, U.S. Department of the Interior, National Park Service.

measured, only mice captured per trap-night. During pretreatment comparison of mice captured per trap-night, no significant difference was observed (Table 4). However, we observed significant differences in 1996-1998 (fewer captures at NASA in 1996 but more in 1997 and 1998 compared to captures at Patuxent), perhaps caused by the small capture numbers, predation, or differences in mast production. There was also evidence of trap invasion and tampering, such as sprung traps most likely caused by squirrels, raccoons, opossums, and deer. Several traps were also located in nearby creeks,

another, though mouse densities per se were not

### Efficacy of deer feeders and acaricide application The deer stations were initially baited with corn in

the feeders. Pieces of apple and corn were also spread

on the stations or nearby on the trails to help attract deer.

traps to small saplings or bushes.

ticks are not presented).

indicating raccoon displacement. Thereafter, we tied the

Once one deer started to feed, other deer were attracted to the stations and also started to feed, at which time the extra bait was discontinued. Our first deer treatment year (1996) resembled the preliminary study reported by Pound et al. (2000) using the acaricide amitraz to selftreat 9 deer in a small-fenced pasture. He reported 92% and 97% control of adult and nymphal A. americanum. Our permethrin efficacy was 78 and 100% for nymphal and adult I. scapularis, and this increased to 100% for all stages in the second year of treatment and remained

demonstrated that ca. 0.2 gm A.I. of permethrin was applied directly to the head, ears, and neck per deer per week by the last year of treatment (Table 7). Pound et al. (2000) also reported that dye applied to the rollers and transferred to the deer was spread from their heads to their rear sides and axillae during self-grooming, thus reaching most of the parasitizing ticks. Since > 90% of

A. americanum adults are found on the head, ears, neck, and chest region of white-tailed deer<sup>2</sup>, and ca. 87% of I. scapularis adults are also found on these areas (Schmidtmann et al. 1998), we expected that area-wide control would occur if the same percentage of I. scapularis adults were killed while feeding and mating on deer over a 3 y treatment period. However, killing all active stages of ticks on deer would theoretically shorten this lag time. Therefore, we applied permethrin during the activity periods of each free-living stage and achieved 100% knockdown of all tick stages parasitizing

mice, while sampled adult questing ticks even increased 3 y after removal of deer. Because deer are good hosts for immature ticks (Piesman and Spielman 1979, Main et al. 1981, Telford et al. 1988), the difference between the Wilson study (1988) and our study presumably was caused by the permethrin-treated deer killing all stages of active ticks. The first group of 5-10 deer feeding at stations newly supplied with permethrin-dye solution applied more permethrin than later deer due to less permethrin-dye being on the rollers, as shown by darker blue-colored ears. To maximize effectiveness of treatment, we initially supplied the solution to the rollers for ~5 d per week during the first month, depending on the weather. During the next 2 d, the feeder troughs were closed. Deer learned rapidly that no feed was present when the plates were

closed. However, if open but empty, the deer pawed the

troughs, knocking the feeders around and even off the

sampled deer by the 2<sup>nd</sup> year of treatment. In addition,

we observed large reductions in all questing ticks and

ticks parasitizing mice. In contrast, in a study on Great

Island, Cape Cod, MA, where 51 of 52 deer were

sacrificed to control ticks, Wilson et al. (1988) showed

a slower decline in larval (mean: ~1 tick/mouse) and

nymphal (mean: ~0.6 ticks/mouse) ticks parasitizing

concrete blocks. During 1997, permethrin was applied 2 times/week, while in 1998 permethrin was applied only twice a week for the 1st week of a 2-week period (Table 7). there for the third year of treatment (data for immature All deer sampled after the stations were in operation in 1995 were darted at a distance at least 175 m from the stations. All of these deer had permethrin-free hair Deer self-application of the permethrin-dye solution samples and dye-free ears, heads, and necks. From 1996 to 1998, a range of 0-9.6 µg permethrin/g hair was detected from the hair samples. The percentages of deer with permethrin were probably higher than shown, since we only took 2 or 3 small samples from the head-neck interface and the ears on one side of the deer. Few bucks appeared to use the stations during the breeding season (rut), but were observed using the stations continuously during spring, summer, and early autumn. A small amount of permethrin on the deer may have been reduced

> by rubbing against underbrush and trees. Rubbing may contribute to permethrin distribution over other parts of

> the deer, to ticks questing on underbrush and tree trunks (Carroll 2002), and to other animals using the same trails.

> Permethrin treatment of other animals, including selftreatment at the stations while eating corn and removal

> of the treated foam from the rollers, may also contribute

to area-wide control of ticks, especially in areas not

<sup>&</sup>lt;sup>2</sup>George, E.J., J.M. Pound, J.A. Miller, D. Fish, G.A. Mound, K. Stafford III, J.F. Carroll, T.L. Schulze, T.J. Daniels, and T.N. Mather. 1997. USDA northeast area-wide tick control project. USDA-ARS, Kerrville, TX.

nests). A comparison can be made of the relative amounts of active ingredient (AI) of pesticide used in controlling ticks with the "4-poster" tick control system of self-

treatment of deer and the area-wide spray treatments on shrubs, leaf litter, and grass currently recommended.

Based on the number of squirrels and other mammals observed eating corn from the deer stations during the daytime, we conservatively estimated that 10% of the

permethrin was used by these small mammals and only 90% was applied by the deer (Table 7). In 1996, 20.6 mg permethrin (AI)/kg deer/week were applied using

the "4-poster," and that treatment was reduced in 1998 to a maintenance dose of 4.12 mg permethrin (AI)/kg deer/wk. Based on the 121 hectare area (deer usable area), an estimated 13 gm permethrin (AI)/hectare (ha)

were applied in 1996 and 5.2 gm/ha were applied in 1998 (Table 7). By comparison, area-wide spray treatment with cyfluthrin, a commonly used pesticide for controlling I. scapularis, was applied at the rate of 410 gm cyfluthrin (AI)/ha (Solberg et al. 1992), and recommended twice a

(AI)/y. Therefore, the self-treatment of deer provided 63 fold annual reduction in pesticide application to the environment in 1996 and a 157-fold annual reduction in 1998 compared to a typical area-wide spray treatment. In addition, the use of the "4-poster" control system confined the pesticide to either the device or the deer and therefore should be more environmentally friendly

Acaricide treatment of deer at NASA should be

year (Stafford 1997), giving a total of 820 gm cyfluthrin

continued to eliminate any surviving residential and immigrating ticks. In addition, new studies are needed to determine if: 1) continued or reduced permethrin application can continue to give the same or better areawide control of I. scapularis populations, 2) the acaricide should be switched at some point to prevent development of permethrin resistance in ticks, and 3) other mammals should be treated at different types of bait stations with

than area-wide spraying.

elimination of all stages of ticks.

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more appropriate bait to expedite and facilitate

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